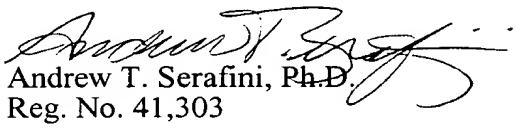
named sequences, SEQ ID NOS:1-252, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Abstract by the current Amendment. The attached pages are captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."**

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 22 of page 14 has been amended as follows:

Figure 15C lists ZFP target sequences (SEQ ID NOS:207, 144 and 240, respectively) and finger designs (SEQ ID NOS:239, 238, 122, 57, 159, 35, 64, 85, 36, 112, 66 and 54, respectively). ZFPs are named according to target site location and the suffix mVZ (for mouse VEGF-A ZFP). Finger designs indicate the identity of amino acid residues at positions -1 to +6 of the alpha helix of each finger.

Paragraph beginning at line 26 of page 14 has been amended as follows:

Figure 15D shows gel-shift assays of binding affinity. A three-fold dilution series of each protein was tested for binding to its DNA target (SEQ ID NOS:207, 144, 240 and 141, respectively), with the highest concentration in lane 10 and the lowest concentration in lane 2. Lane 1 contains probe alone. Apparent K_d's, derived from the average of 3 such studies, are indicated at right. For mVZ+426 and mVZ+509, K_d's are provided as upper bounds (<0.01 nM), since the use of 0.01 nM of probe has probably led to an underestimate of the affinity of these proteins.

Paragraph beginning at line 20 of page 17 has been amended as follows:

The term "zinc finger protein" or "ZFP" refers to a protein having DNA binding domains that are stabilized by zinc. The individual DNA binding domains are typically referred to as "fingers" A ZFP has least one finger, typically two, three, four, five, six or more fingers. Each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A ZFP binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid,

zinc-chelating, DNA-binding subdomain. An exemplary motif characterizing one class of these proteins (C_2H_2 class) is -Cys-$(X)_{2-4}$-Cys-$(X)_{12}$-His-$(X)_{3-5}$-His (SEQ ID NO:208) (where X is any amino acid). Additional classes of zinc finger proteins are known and are useful in the practice of the methods, and in the manufacture and use of the compositions disclosed herein (see, e.g., Rhodes et al. (1993) Scientific American 268:56-65). Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues coordinated with zinc along with the two cysteine residues of a single beta turn (*see, e.g.*, Berg & Shi, *Science* 271:1081-1085 (1996)).

Paragraph beginning at line 3 of page 34 has been amended as follows:

The zinc finger proteins (ZFPs) disclosed herein are proteins that can bind to DNA in a sequence-specific manner. As indicated supra, these ZFPs can be used in a variety of applications, including modulating angiogenesis and in treatments for ischemia. An exemplary motif characterizing one class of these proteins, the C_2H_2 class, is -Cys-$(X)_{2-4}$-Cys-$(X)_{12}$-His-$(X)_{3-5}$-His (SEQ ID NO:208) (where X is any amino acid) [(SEQ. ID. NO:___)]. Several structural studies have demonstrated that the finger domain contains an alpha helix containing the two invariant histidine residues and two invariant cysteine residues in a beta turn coordinated through zinc. However, the ZFPs provided herein are not limited to this particular class. Additional classes of zinc finger proteins are known and can also be used in the methods and compositions disclosed herein (see, e.g., Rhodes, et al. (1993) Scientific American 268:56-65). In certain ZFPs, a single finger domain is about 30 amino acids in length. Zinc finger domains are involved not only in DNA-recognition, but also in RNA binding and in protein-protein binding.

Paragraph beginning at line 3 of page 36 has been amended as follows:

Tables 3 and 4 show the amino acid sequences of a number of different ZFPs and the corresponding target sites to which they bind. Table 3 lists ZFPs that bind to target sites that include 9 nucleotides. The first column in this table lists an internal

reference name of the ZFP. Column 2 includes the 9 base target site bound by a three-finger zinc finger protein, with the target sites listed in 5' to 3' orientation. The corresponding SEQ ID NO: [SEQ ID NO.] for the target site is listed in column 3 (SEQ ID NOS:1-29 and 244). The amino acid sequences of portions of the three zinc finger components involved in recognition are listed in columns 4, 6 and 8, and their corresponding SEQ ID NOS: [SEQ ID NOs]. are listed in columns 5 (SEQ ID NOS:30-58) , 7 (SEQ ID NOS:59-87, 112, and 245-252) and 9 (SEQ ID NOS:42, 64, and88-116), respectively. The numbering convention for zinc fingers is defined below. Column 10 lists the dissociation constants for some of the ZFP/target site complexes. Methods for determining such constants are described *infra*. Excluding cross-strand interactions, each finger binds to a triplet of bases (a target subsite) within a corresponding target sequence. The first finger binds to the first triplet starting from the 3' end of a target site, the second finger binds to the second triplet, and the third finger binds the third (i.e., the 5'-most) triplet of the target sequence. Thus, for example, the RSDHLAR finger (SEQ ID NO:30) [(SEQ ID NO:___)]of the ZFP BVO 13A (first column of Table 3) binds to 5'GGG3', the DRSNLTR finger (SEQ ID NO:59) [(SEQ ID NO: ___)] binds to 5'GAC3' and the RSDALTQ finger (SEQ ID NO:88) [(SEQ ID NO:__)] binds to 5'ATG3'.

Paragraph beginning at line 20 of page 36 has been amended as follows:

Table 4 provides information on six-finger ZFPs targeting VEGF genes. Table 4 has a similar format to Table 3, with column 1 indicating the internal reference name of the ZFP. In contrast to Table 3, however, column 2 of Table 4 includes the 18 base target site recognized by a six-finger protein (here, too, targets are listed in a 5' to 3' orientation), with the corresponding SEQ ID NO: [SEQ ID NO.] listed in column 3 (SEQ ID NOS:117-119). The amino acid sequences of portions of the six zinc finger components involved in recognition are listed in columns 4, 6, 8, 10, 12 and 14, with associated SEQ ID NOS: [SEQ ID NOs.] being listed in columns 5 (SEQ ID NOS:120-122), 7 (SEQ ID NOS:123-125), 9 (SEQ ID NOS:126-128), 11 (SEQ ID NOS:129-131), 13 (SEQ ID NOS:132-134) and 15 (SEQ ID NOS:135-17), respectively. In ZFPs of this type, the first finger binds to the first triplet starting from the 3' end of a target site, the

second finger binds to the second triplet, the third finger binds the third triplet, the fourth finger binds to the fourth triplet, the fifth finger binds to the fifth triplet and the sixth finger binds to the sixth (i.e., the 5'-most) triplet of the target sequence (again excluding cross-strand interactions). Hence, for the ZFP named BVO 10A-9A, the first finger QSSDLRR (SEQ ID NO:120) [(SEQ ID NO:__)] binds 5'GCT3', the second finger RSDHLTR (SEQ ID NO:123) [(SEQ ID NO:__)] binds 5'GGG3', the third finger DRSALAR (SEQ ID NO:126) [(SEQ ID NO:__)] binds 5'GTC3', the fourth finger RSDHLAR (SEQ ID NO:129) [(SEQ ID NO: -_)] binds 5'GGG3', the fifth finger RSDNLAR (SEQ ID NO:132) [(SEQ ID NO:__)] binds 5'GAG3' and the sixth finger RSDALTR (SEQ ID NO:135) [(SEQ ID NO:__)] binds 5'GTG3'.

Paragraph beginning at line 26 of page 38 has been amended as follows:

The relative order of fingers in a zinc finger protein from N-terminal to C-terminal determines the relative order of triplets in the 3' to 5' direction in the target. For example, if a zinc finger protein comprises from N-terminal to C-terminal first, second and third fingers that individually bind, respectively, to triplets 5' GAC3', 5'GTA3' and 5"GGC3' then the zinc finger protein binds to the target segment 3'CAGATGCGG5' (SEQ ID NO:209) [(SEQ ID NO: ___)]. If the zinc finger protein comprises the fingers in another order, for example, second finger, first finger, third finger, then the zinc finger protein binds to a target segment comprising a different permutation of triplets, in this example, 3'ATGCAGCGG5' (SEQ ID NO:210) [(SEQ ID NO: ___)]. See Berg & Shi, *Science* 271, 1081-1086 (1996). The assessment of binding properties of a zinc finger protein as the aggregate of its component fingers may, in some cases, be influenced by context-dependent interactions of multiple fingers binding in the same protein.

Paragraph beginning at line 16 of page 39 has been amended as follows:

Linkage can be accomplished using any of the following peptide linkers. T G E K P (SEQ ID NO:211),: [(SEQ ID NO:___)] (Liu et al., 1997, supra.); $(G_4S)n$ (SEQ ID NO:212), [(SEQ ID NO:___)] (Kim et al., Proc. Natl. Acad. Sci. U.S.A. 93: 1156-

1160 (1996.); GGRRGGGS (SEQ ID NO:213); [(SEQ ID NO:____)] LRQRDGERP (SEQ ID NO:214); [(SEQ ID NO:____)] LRQKDGGGSERP (SEQ ID NO:215); [(SEQ ID NO:___)] LRQKD$(G_3S)_2$ERP (SEQ ID NO:216). [(SEQ ID NO:__)] Alternatively, flexible linkers can be rationally designed using computer programs capable of modeling both DNA-binding sites and the peptides themselves or by phage display methods . In a further variation, noncovalent linkage can be achieved by fusing two zinc finger proteins with domains promoting heterodimer formation of the two zinc finger proteins. For example, one zinc finger protein can be fused with fos and the other with jun (see Barbas et al., WO 95/119431).

Paragraph beginning at line 31 of page 39 has been amended as follows:

A component finger of zinc finger protein typically contains about 30 amino acids and, in one embodiment, has the following motif (N-C) (SEQ ID NO:208):

[(SEQ ID NO:__)]

$Cys-(X)_{2-4}-Cys-X.X.X.X.X.X.X.X.X.X.X.X-His-(X)_{3-5}-His$

-1 1 2 3 4 5 6 7

Paragraph beginning at line 14 of page 40 has been amended as follows:

The ZFPs provided herein are engineered to recognize a selected target site in a VEGF gene such as shown in Tables 3, 4 and 6. The process of designing or selecting a ZFP typically starts with a natural ZFP as a source of framework residues. The process of design or selection serves to define nonconserved positions (i.e., positions -1 to +6) so as to confer a desired binding specificity. One suitable ZFP is the DNA binding domain of the mouse transcription factor Zif268. The DNA binding domain of this protein has the amino acid sequence:

YACPVESCDRRFSRSDELTRHIRIHTGQKP (F1) (SEQ ID NO:217) [(SEQ ID NO:____)]

FQCRICMRNFSRSDHLTTHIRTHTGEKP (F2) (SEQ ID NO:218) [(SEQ ID NO:____)]

FACDICGRKFARSDERKRHTKIHLRQK (F3) (SEQ ID NO:219) [SEQ ID NO:_____)]

and binds to a target 5' GCG TGG GCG 3' (SEQ ID NO:220) [(SEQ ID NO:_____)].

Paragraph beginning at line 25 of page 40 has been amended as follows:

Another suitable natural zinc finger protein as a source of framework residues is Sp-1. The Sp-1 sequence used for construction of zinc finger proteins corresponds to amino acids 531 to 624 in the Sp-1 transcription factor. This sequence is 94 amino acids in length. The amino acid sequence of Sp-1 is as follows:
PGKKKQHICHIQGCGKVYGKTSHLRAHLRWHTGERPFMCTWSYCGKRFTRSDEL
QRHKRTHTGEKKFACPECPKRFMRSDHLSKHIKTHQNKKG (SEQ ID NO:221)
[(SEQ ID NO:___)]
Sp-1 binds to a target site 5'GGG GCG GGG3' (SEQ ID NO:222) [(SEQ ID No: 14)].

Paragraph beginning at line 32 of page 40 has been amended as follows:

An alternate form of Sp-1, an Sp-1 consensus sequence, has the following amino acid sequence:
meklmgsgdPGKKKQHACPECGKSFSKSSHLRAHQRTHTGERPYKCPECGKSFSRSD
ELQRHQRTHTGEKPYKCPECGKSFSRSDHLSKHQRTHQNKKG (SEQ ID NO:223)
[(SEQ ID NO:___)] (lower case letters are a leader sequence from Shi & Berg, *Chemistry and Biology* 1, 83-89. (1995). The optimal binding sequence for the Sp-1 consensus sequence is 5'GGGGCGGGG3' (SEQ ID NO:222) [(SEQ ID NO:___)]. Other suitable ZFPs are described below.

Paragraph beginning at line 22 of page 74 has been amended as follows:

Construction of Zinc Finger Fusion Proteins. VEGF-A-targeted zinc fingers were assembled in an SP1 backbone and cloned into the pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA) as described previously (Zhang et al., supra; WO 00/41566; and WO 00/42219). A CMV promoter was used to drive the expression of all the ZFPs in mammalian cells. All ZFP constructs contained an N-terminal nuclear

localization signal (Pro-Lys-Lys-Lys-Arg-Lys-Val; SEQ ID NO:224,[SEQ ID NO: _____]) from SV40 large T antigen, a Zinc Finger DNA-binding domain, an activation domain, and a FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys; SEQ ID NO:225, [SEQ ID NO: _____]). ZFP-VP16 fusions contained the herpes simplex virus VP16 activation domain from amino acid 413 to 490 (Sadowski et al., supra; Zhang et al, supra; WO 00/41566; and WO 00/42219). ZFP-p65 fusions contained the human NF-κB transcription factor p65 subunit (amino acid 288-548) as the activation domain (Ruben et al., supra).

Paragraph (TABLE 5) beginning at line 22 of page 74 has been amended as follows:

TABLE 5: NUCLEOTIDE SEQUENCES OF PRIMERS AND PROBES USED FOR TAQMAN ANALYSIS

	Sequence	SEQ ID NO:
VEGF-A forward primer	5'-GTGCATTGGAGCCTTGCCTTG-3'	226
VEGF-A reverse primer	5'-ACTCGATCTCATCAGGGTACTC-3'	227
VEGF-A Taqman Probe	5'-FAM-CAGTAGCTGCGCTGATAGACATCCA-TAMRA-3'	228
GAPDH forward primer	5'-CCATGTTCGTCATGGGTGTGA-3'	229
GAPDH reverse primer	5'-CATGGACTGTGGTCATGAGT-3'	230
GAPDH Taqman Probe	5'-FAM-TCCTGCACCACCAACTGCTTAGCA-TAMRA-3'	231
VP16-FLAG forward primer	5'-CATGACGATTTCGATCTGGA-3'	232
VP16-FLAG reverse primer	5'-CTACTTGTCATCGTCGTCCTTG-3'	233
VP16-FLAG Taqman Probe	5'-FAM-ATCGGTAAACATCTGCTCAAACTCGA-TAMRA-3'	234

Abbreviations: FAM: aminofluorescein; TAMRA: tetramethylrhodamine

Paragraph beginning at line 1 of page 78 has been amended as follows:

Analysis of splice variants of VEGF-A mRNA - To detect the multiple splice variants of VEGF-A mRNA, total RNA samples (0.5 μg) were subjected to a 20-cycle RT-PCR reaction using Titan™ one-tube RT-PCR system (Roche Molecular Biochemicals, Indianapolis, IN). The primers used were 5'-ATGAACTTTCTGCTGTCTTGGGTGCATT-3' (SEQ ID NO:235) [(SEQ ID

follows: (i) PCR of the '4-6' ZFP gene using the primers 5' CCCAGATCTGGTGATGGCAAGAAGAAGCAGCACCATCTGCCACATCCAG (SEQ ID NO:241) [(SEQ ID NO: ____]and 5' CCCAAGCTTAGGATCCACCCTTCTTGTTCTGGTGGGT (SEQ ID NO:242) [(SEQ ID NO:___]; (ii) digestion of the resultant fragment with Bgl II and Hind III (sites underlined in primers); and (iii) ligation into the BamHI and Hind III sites of the pMal-c2 '1-3'. The resultant protein, VZ+57, consists of the '1-3' and '4-6' three-finger modules connected by a flexible peptide linker, with the amino acid sequence between the second zinc-coordinating histidine of finger 3 and the first zinc-coordinating cysteine of finger 4 (both underlined) as follows: HQNKKGGSGDGKKKQHIC (SEQ ID NO:243).

Paragraph beginning at line 15 of page 90 has been amended as follows:

Construction of retroviral vectors. The retroviral vectors described here are derived from a pLXSN, a Moloney murine leukemia virus-based vector containing a neomycin resistance gene under the control of an internal simian virus (SV40) promoter. Using EcoR1 and Xho1 restriction sites, the zinc finger expression cassette was placed immediately downstream of the LTR in pLXSN. Briefly, all ZFP constructs contained an N-terminal nuclear localization signal (Pro-Lys-Lys-Lys-Arg-Lys-Val; SEQ ID NO:224) from SV40 largeT antigen, a Zinc Finger DNA-binding domain, the herpes simplex virus VP16 activation domain from amino acid 413 to 490, and a FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys; SEQ ID NO:224). The LXSN vectors were produced in the 293 AMPHO-PAK™ cell line and had titers ranging from 0.5-1.0 x 10^6 G418-resistant colony-forming units. Virus-containing supernatant was collected 48 hr after transfection, filtered through 0.45-mm-pore-size filter and used fresh for transduction of target cells or aliquoted and stored at –80 °C.

Paragraph (**TABLE 3**) beginning at line 1 of page 104 has been amended as follows:

VOP 35A-10	GCTGGAGCA	28	QSGSLTR	57	QSGHLQR	86	QSSDLTR	115	<.02
ZEN-7A 1	GGGGGHGCT	29	QSSDLRR	58	QSSHLAR	87	RSDHLSR	116	.63
VOP 29A-3	GAGGCTTGG	244	RSDHLTT	51	QSSDLTR	112	RSDNLTR	42	<.02
VOP 32-C	GGGGGTGAC	26	DRSNLTR	31	TSGHLTR	245	RSDHLSR	68	ND
VOP 32-D	GGGGGTGAC	26	DRSNLTR	36	TSGHLIR	246	RSDHLSR	68	ND
VOP 32-E	GGGGGTGAC	26	DRSNLTR	36	TSGHLSR	247	RSDHLSR	68	ND
VOP 32-F	GGGGGTGAC	26	DRSNLTR	36	TSGHLAR	248	RSDHLSR	68	ND
VOP 32-G	GGGGGTGAC	26	DRSNLTR	36	TSGHLRR	249	RSDHLSR	68	ND
VOP 32-H	GGGGGTGAC	26	DRSNLTR	36	TAGHLVR	250	RSDHLSR	68	ND
VOP 32-I	GGGGGTGAC	26	DRSNLTR	36	TTGHLVR	251	RSDHLSR	68	ND
VOP 32-J	GGGGGTGAC	26	DRSNLTR	36	TKDHLVR	252	RSDHLSR	68	ND

103 Paragraph (**TABLE 7**) beginning at line 1 of page 107 has been amended as follows:

TABLE 7 Target sites and recognition helix sequences of rat VEGF-targeted ZFPs

ZFP NAME	TARGET	LOCATION	RECOGNITION HELICES
BV0 12A- 11A	GGAGAGGGGGCCGCAGTG (SEQ ID NO: 182)	+785	F1: RSDALTR (SEQ ID NO:[ ]186) F2: QSGDLTR (SEQ ID NO:[ ]187) F3: ERGDLTR (SEQ ID NO:[ ]188) F4: RSDHLAR (SEQ ID NO:[ ]189) F5: RSDNLAR (SEQ ID NO:[ ]190) F6: QSSHLAR (SEQ ID NO:[ ]191)
BVO 14A- 13B	ATGGACGGGtGAGGCGGCG (SEQ ID NO: 183)	+830	F1: RSDELTR (SEQ ID NO:[ ]192) F2: RSDELQR (SEQ ID NO:[ ]193) F3: RSDNLAR (SEQ ID NO:[ ]194) F4: RSDHLAR (SEQ ID NO:[ ]195) F5: DRSNLTR (SEQ ID NO:[ ]196) F6: RSDALTQ (SEQ ID NO:[ ]197)
VOP 32A	GGGGGTGAC (SEQ ID NO: 184)	+420	F1: DRSNLTR (SEQ ID NO:[]198) F2: MSHHLSR (SEQ ID NO:[ ]199) F3: RSDHLSR (SEQ ID NO:[ ]200)
VOP 30A	GCTGGGGGC (SEQ ID NO: 185)	+40 +514	F1: DRSHLTR (SEQ ID NO:[ ]201) F2: RSDHLTR (SEQ ID NO:[ ]202) F3: QSSDLTR (SEQ ID NO:[ ]203)
VOP 32B	GGGGGTGAC (SEQ ID NO:26)	+420	F1: DRSNLTR (SEQ ID NO:36) F2: TSGHLVR (SEQ ID NO:168) F3: RSDHLSR (SEQ ID NO:64)